Photothermal Chemistry of Collagen During Mid-IR Laser Ablation

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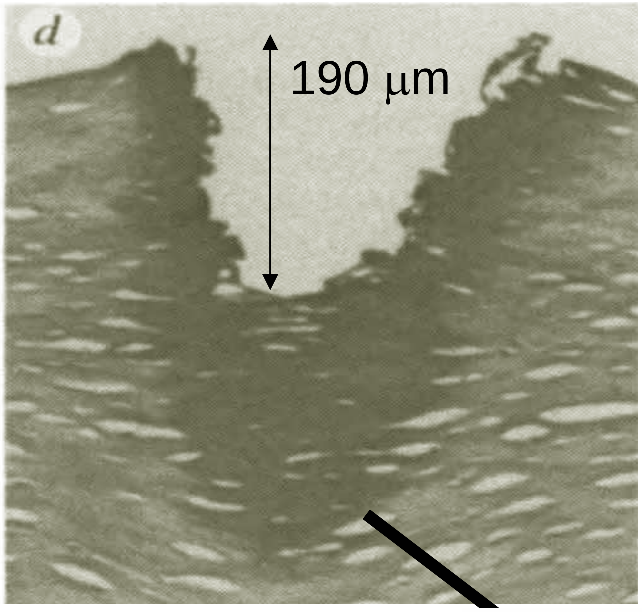
Meek et al, 2001.

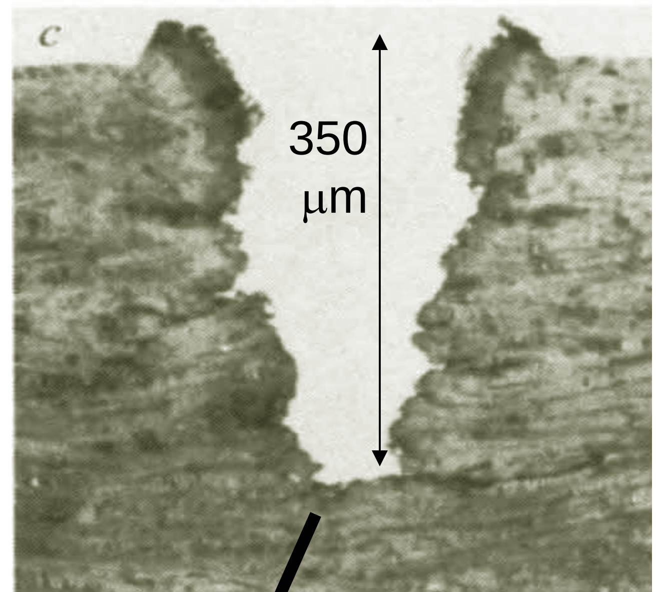
To remove tissue with a laser, protein matrix must lose its structural integrity.

How does failure occur?

Can you influence it by your choice of laser parameters (λ, τ, Φ)?

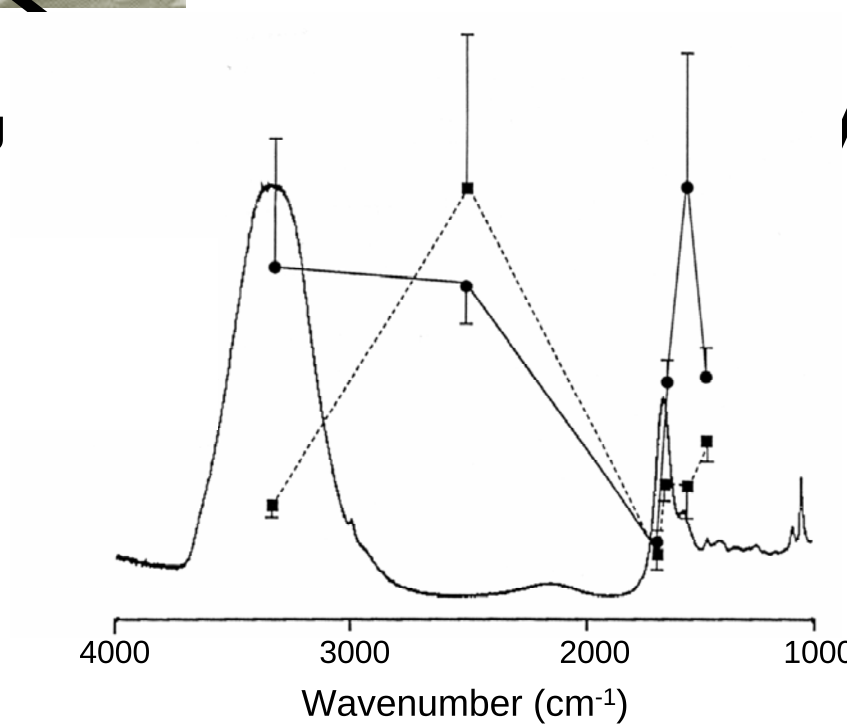


Corneas exposed to 10 macropulses at 4 Hz.

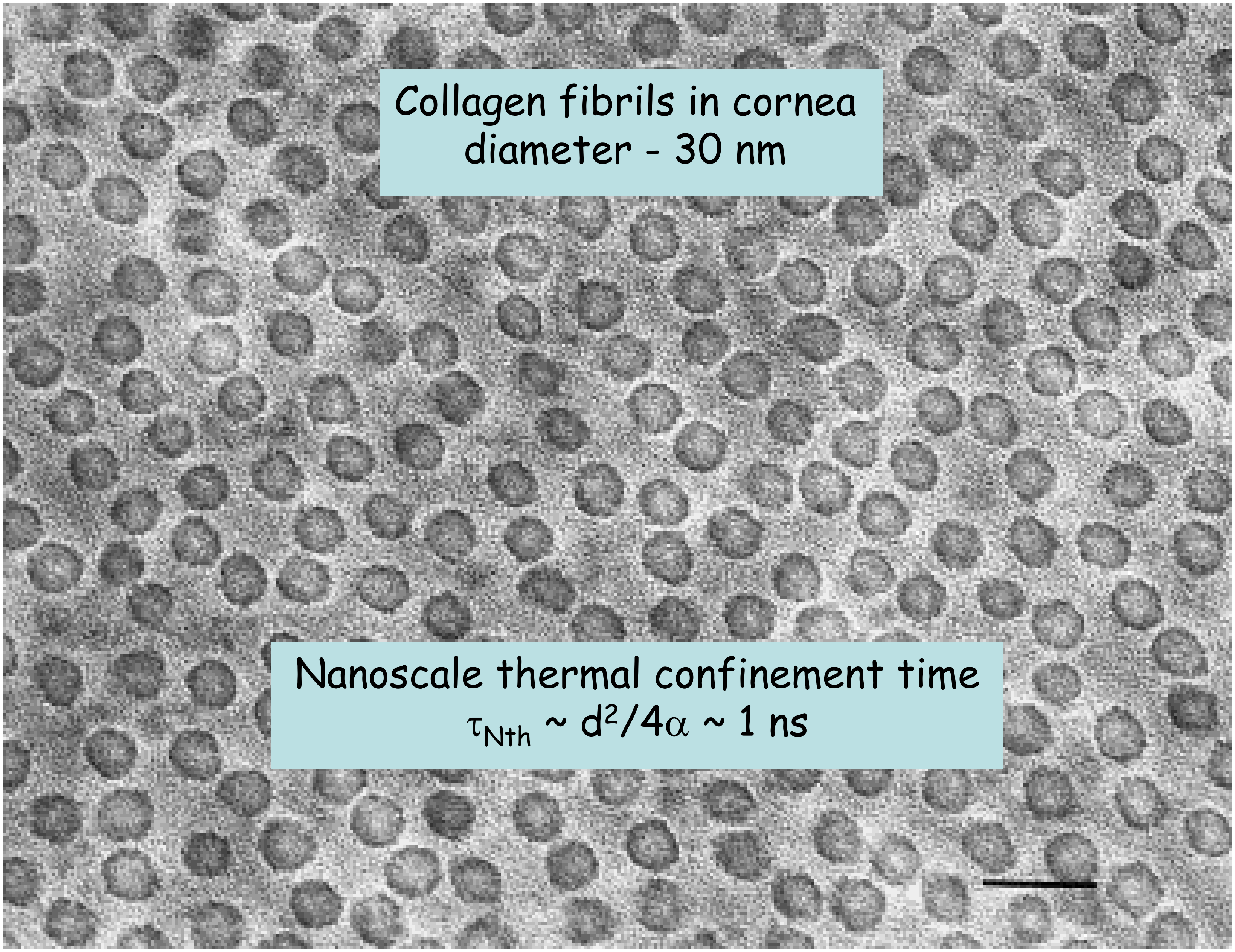


λ= 3.0 μm
E_{macro} = 30 mJ

λ= 6.45 μm
E_{macro} = 20 mJ

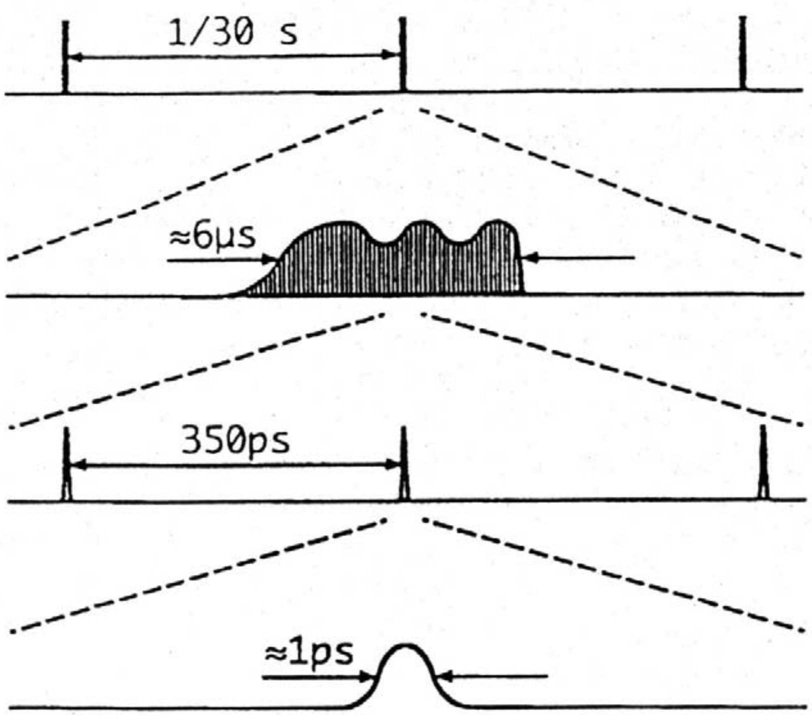


G. Edwards et al (1994)
Nature 371: 416-419.



Meek et al, 2001.

Vanderbilt FEL Pulse Structure



P_{avg} = 0.6 W, I_{avg} = 7.2 x 10^3 W/cm^2

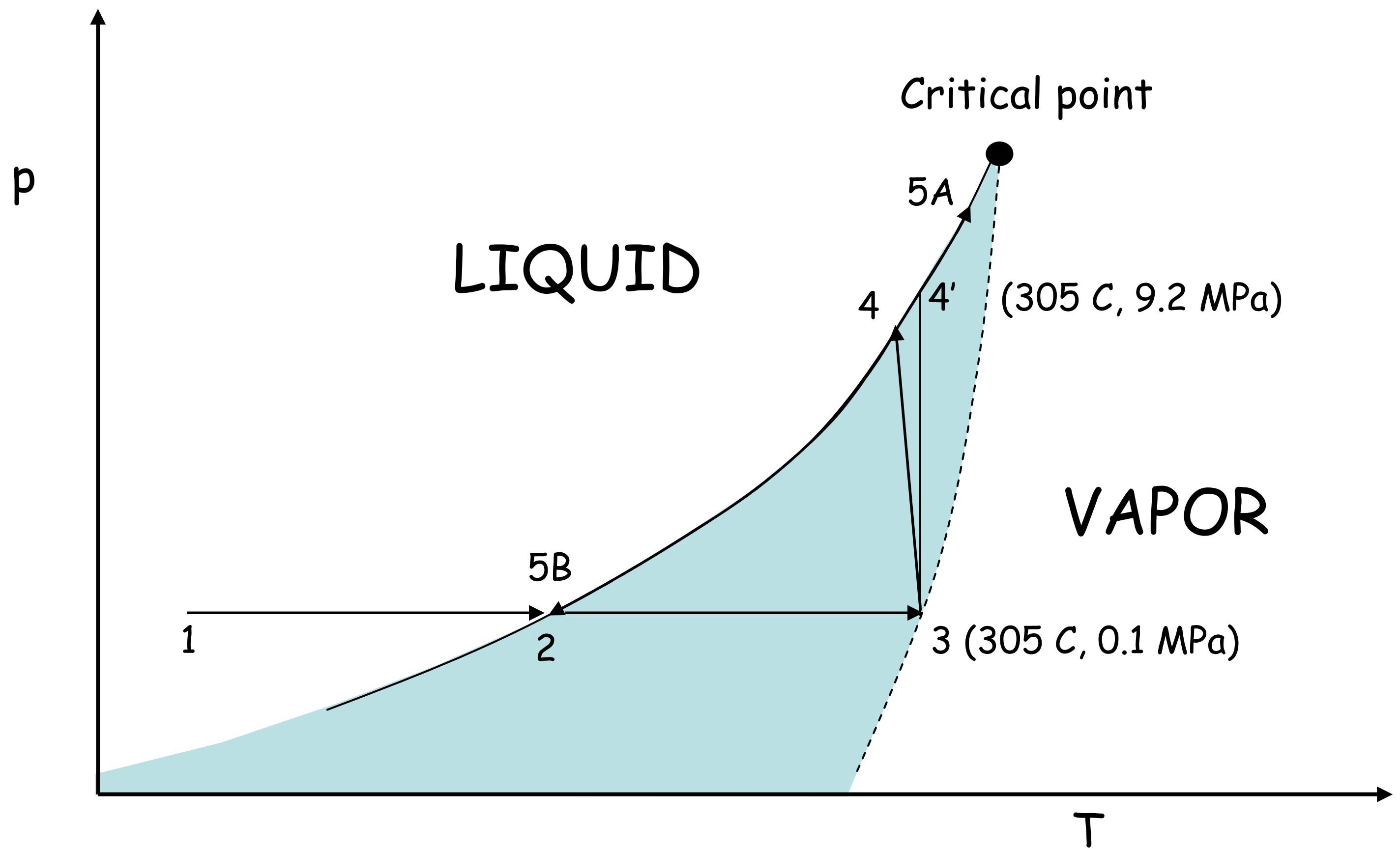
E_{macro} = 20 mJ, **Φ_{macro} = 240 J/cm^2**

P_{macro} = 3.3 kW, I_{macro} = 4.0 x 10^7 W/cm^2

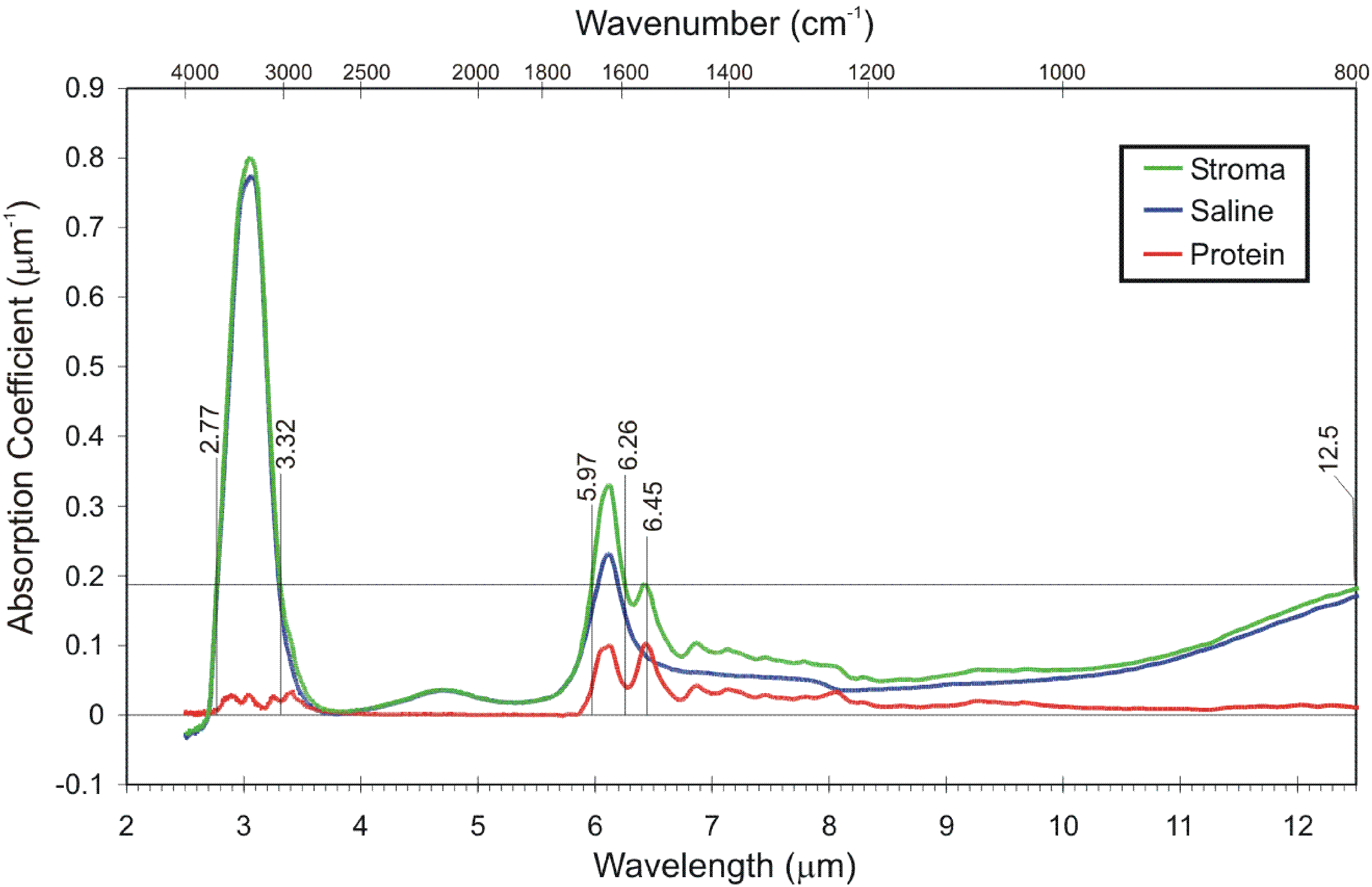
E_{ps} = 1.2 μJ, Φ_{ps} = 0.014 J/cm^2

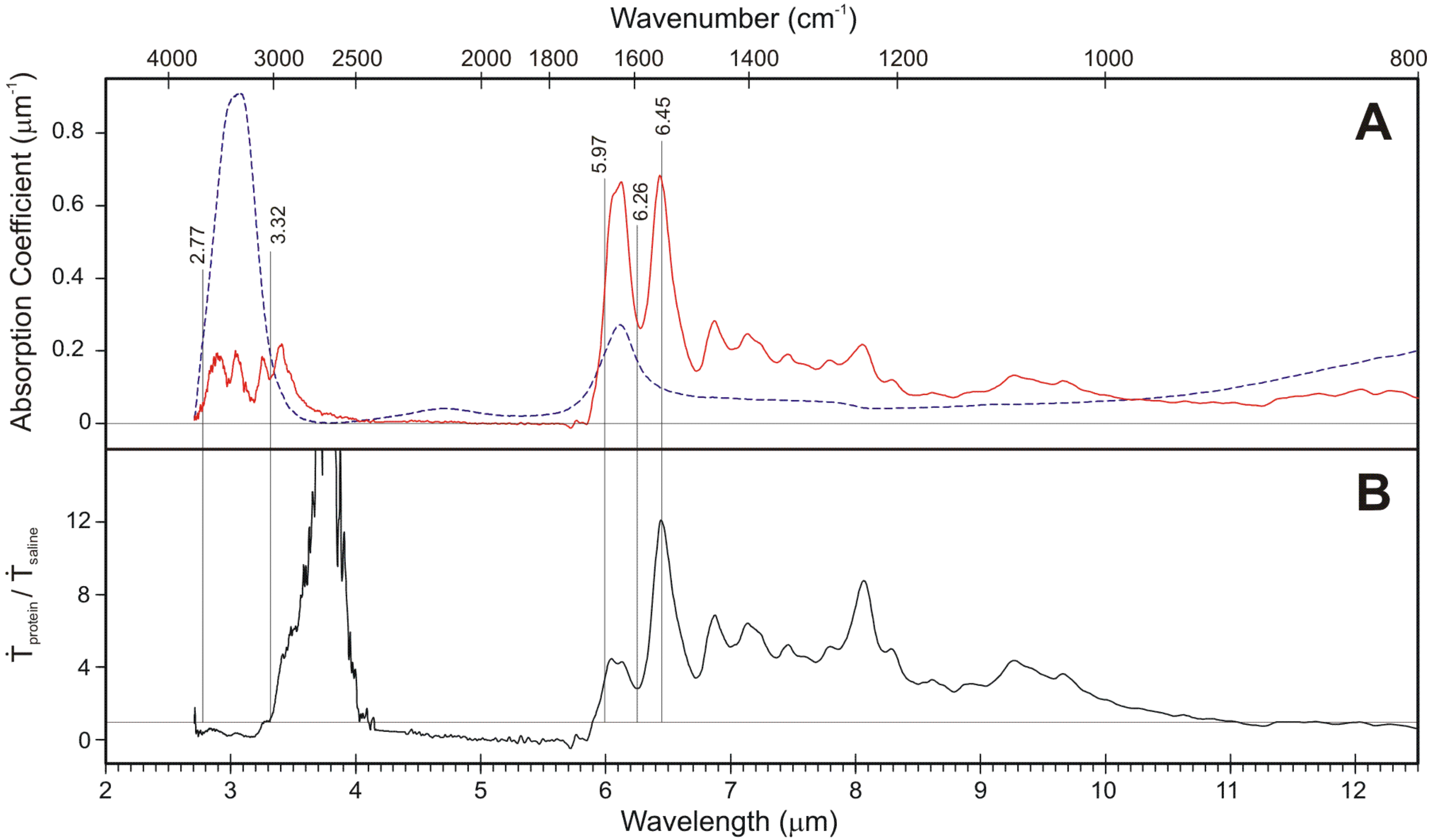
P_{ps} = 1.2 MW, I_{ps} = 1.4 x 10^{10} W/cm^2

Explosive Vaporization



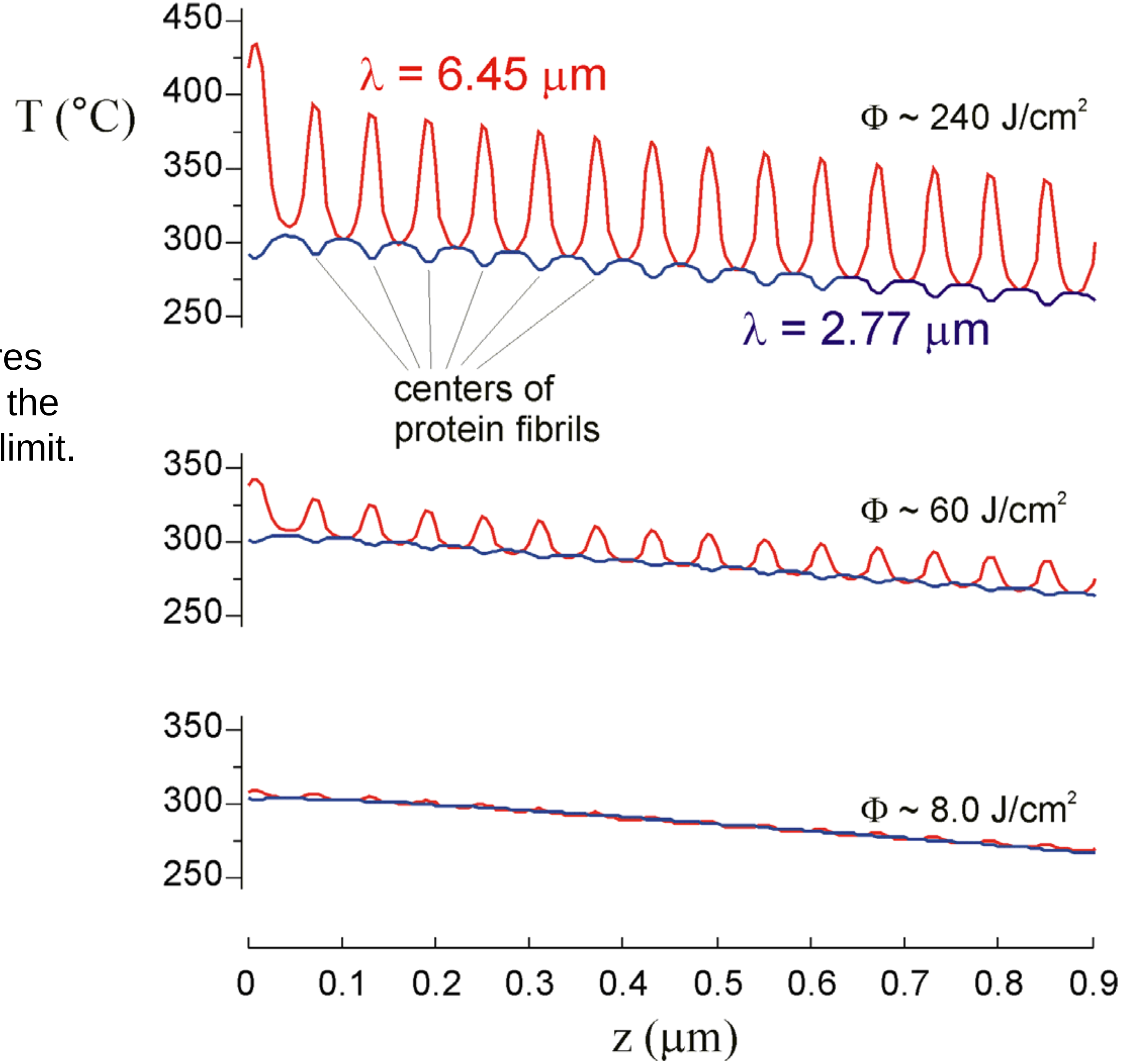
A. Vogel and V. Venugopalan (2003), Chem. Rev. 103: 577-644.


Wavenumber (cm-1)
4000
3000
2500
2000
1800
1600
1400
1200
1000
800
Stroma
Saline
Protein
Absorption Coefficient (µm-1)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
2.77
3.32
5.97
6.26
6.45
12.5
2
3
4
5
6
7
8
9
10
11
12
Wavelength (µm)



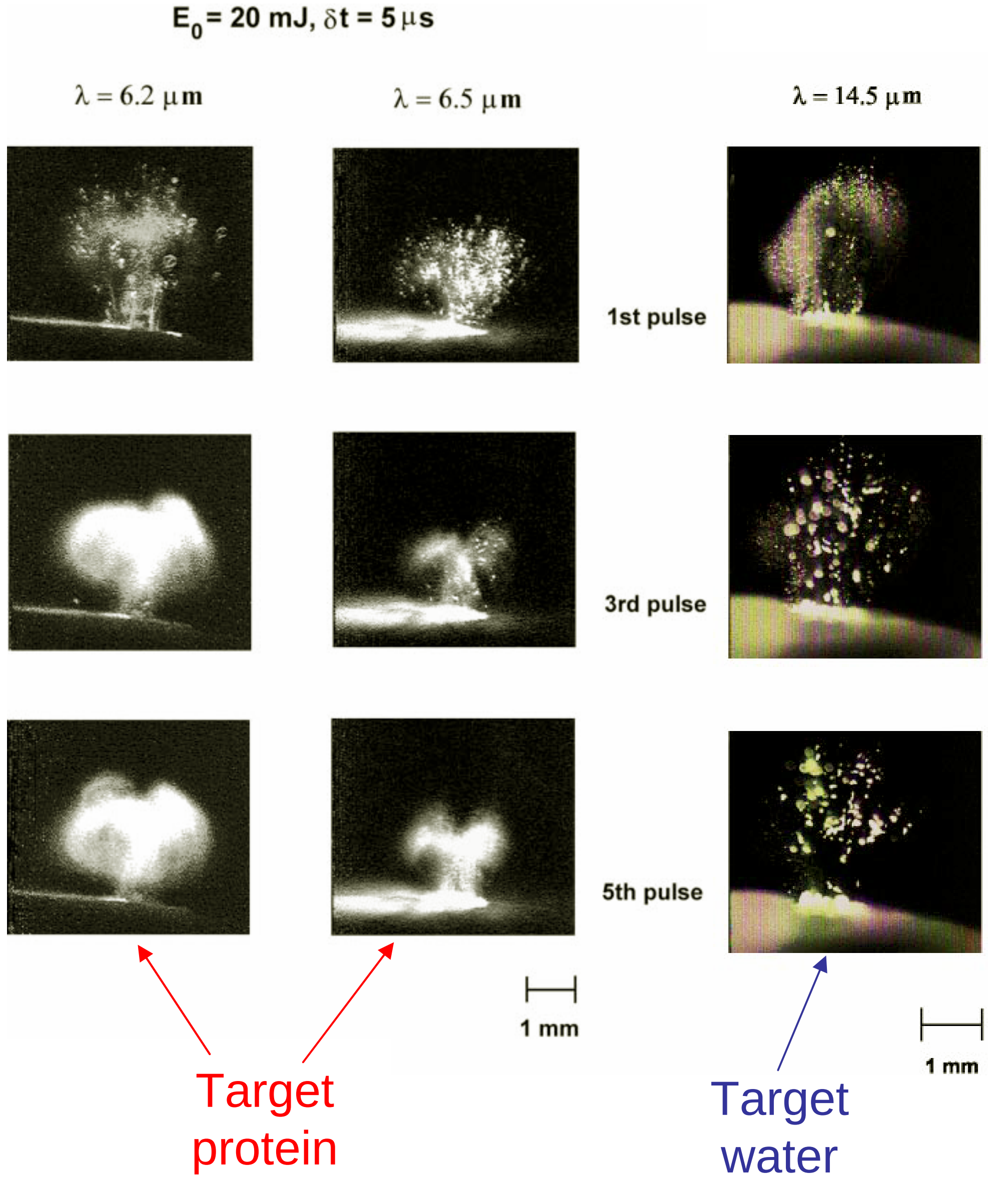
Wavenumber (cm^{-1})
4000
3000
2500
2000
1800
1600
1400
1200
1000
800
A
B
Absorption Coefficient (μm^{-1})
0.8
0.6
0.4
0.2
0
2.77
3.32
5.97
6.26
6.45
$\dot{T}_{protein} / \dot{T}_{saline}$
12
8
4
0
2
3
4
5
6
7
8
9
10
11
12
Wavelength (μm)

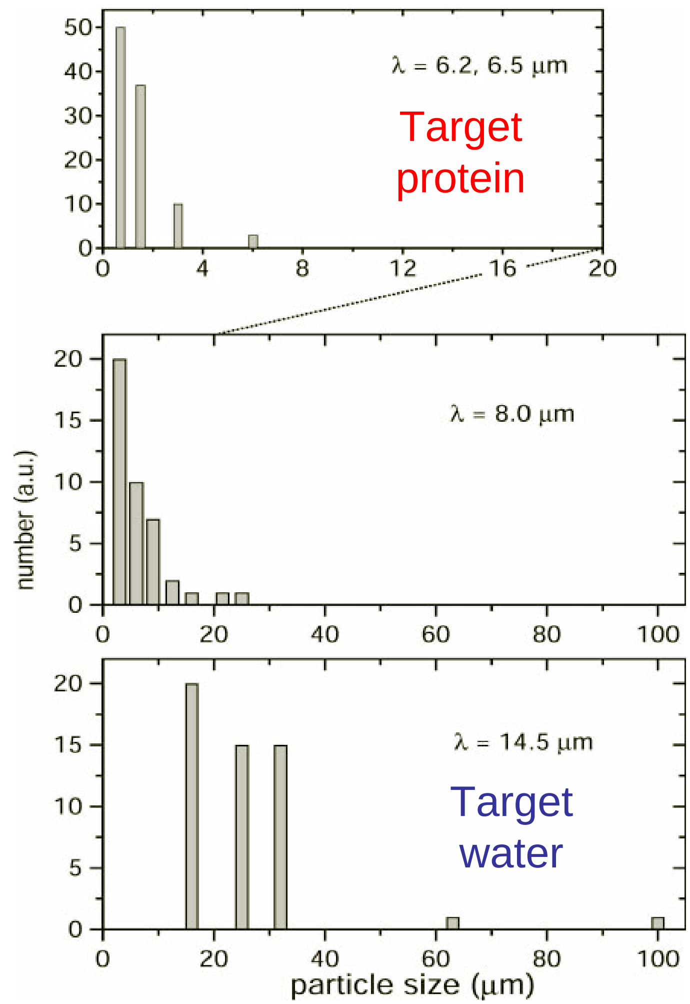
Predicted temperatures attained at the superheat limit.

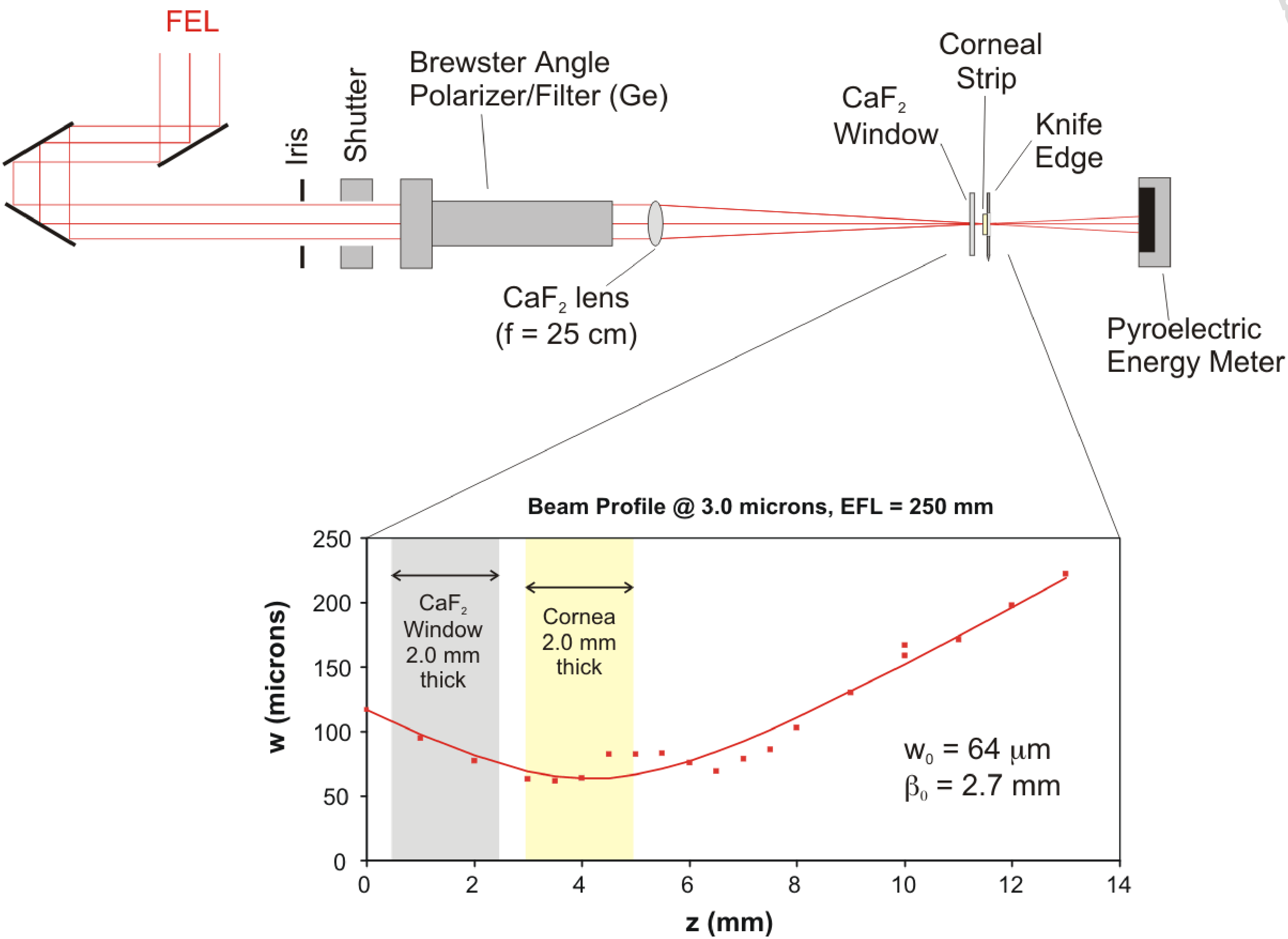


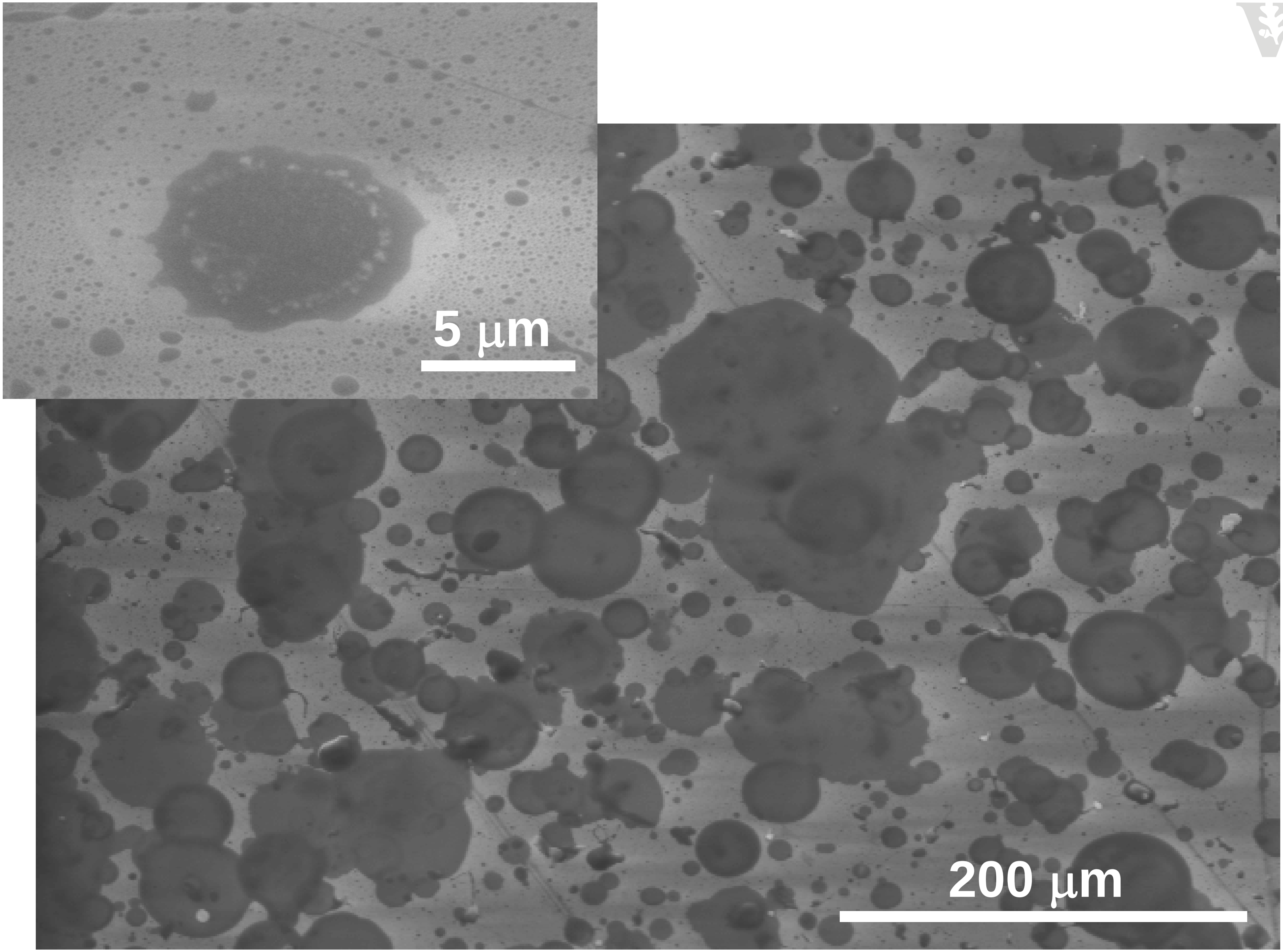
Data from FELIX

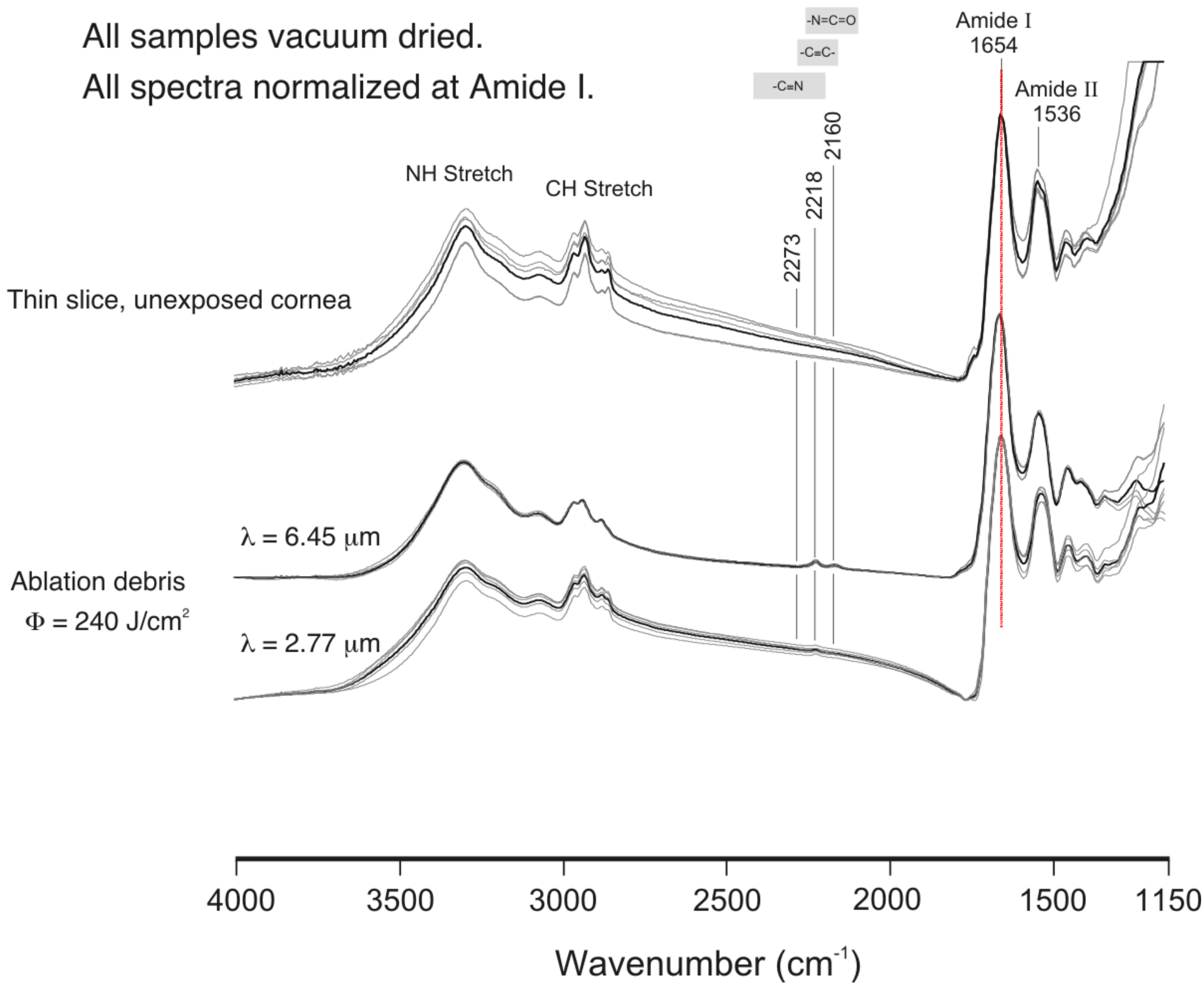
J.M. Auerhammer et al (1999) *Appl. Phys. B* 68: 111–119.





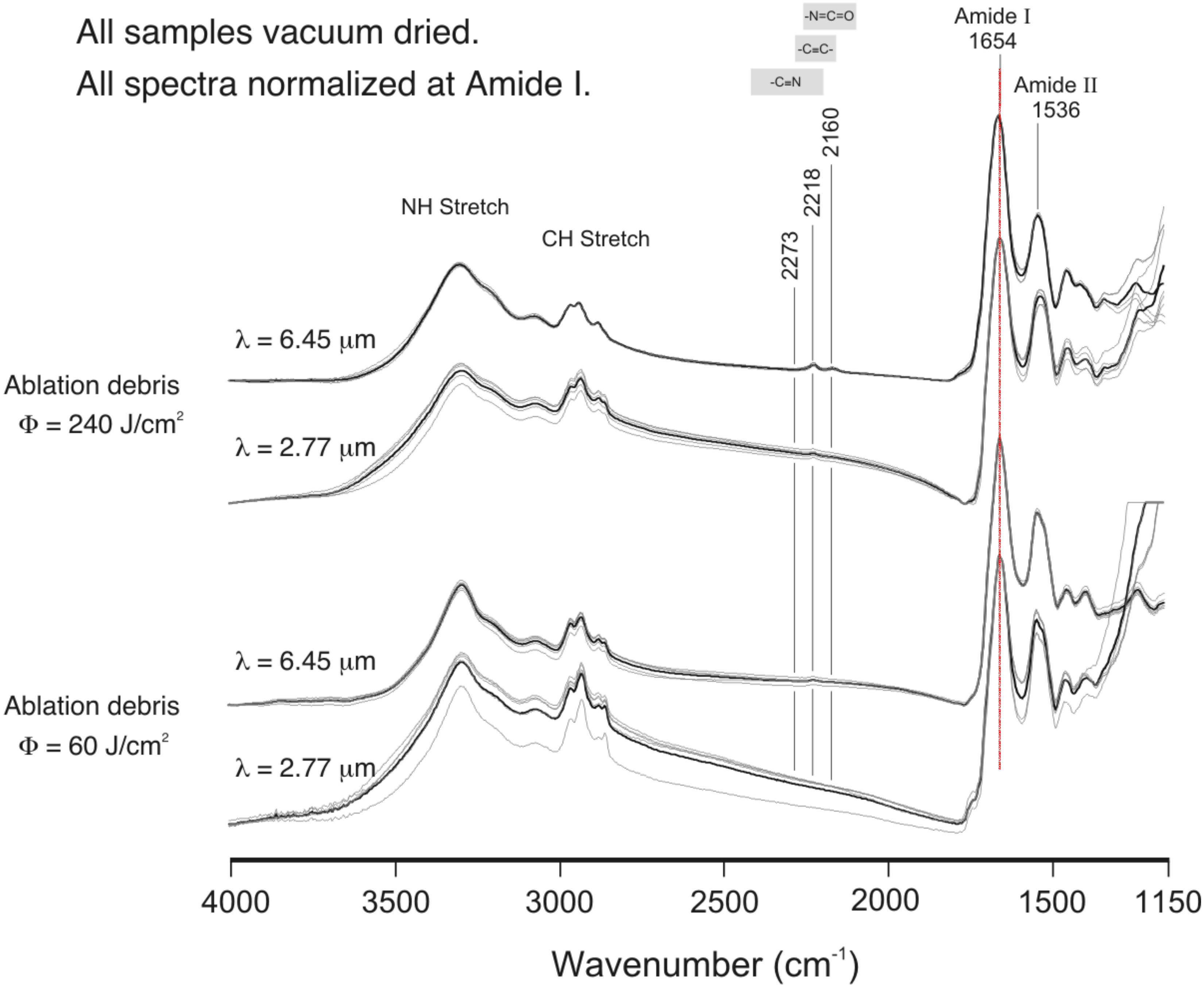

FEL
Iris
Shutter
Brewster Angle
Polarizer/Filter (Ge)
CaF2 lens
(f = 25 cm)
CaF2
Window
Corneal
Strip
Knife
Edge
Pyroelectric
Energy Meter
Beam Profile @ 3.0 microns, EFL = 250 mm
CaF2
Window
2.0 mm
thick
Cornea
2.0 mm
thick
w0 = 64 μm
β0 = 2.7 mm
w (microns)
z (mm)
0
50
100
150
200
250
0
2
4
6
8
10
12
14


5 µm
200 µm


All samples vacuum dried.
All spectra normalized at Amide I.
-N=C=O
-C≡C-
-C≡N
Amide I
1654
Amide II
1536
2160
2218
2273
NH Stretch
CH Stretch
Thin slice, unexposed cornea
λ = 6.45 μm
Ablation debris
Φ = 240 J/cm²
λ = 2.77 μm
4000
3500
3000
2500
2000
1500
1150
Wavenumber (cm⁻¹)

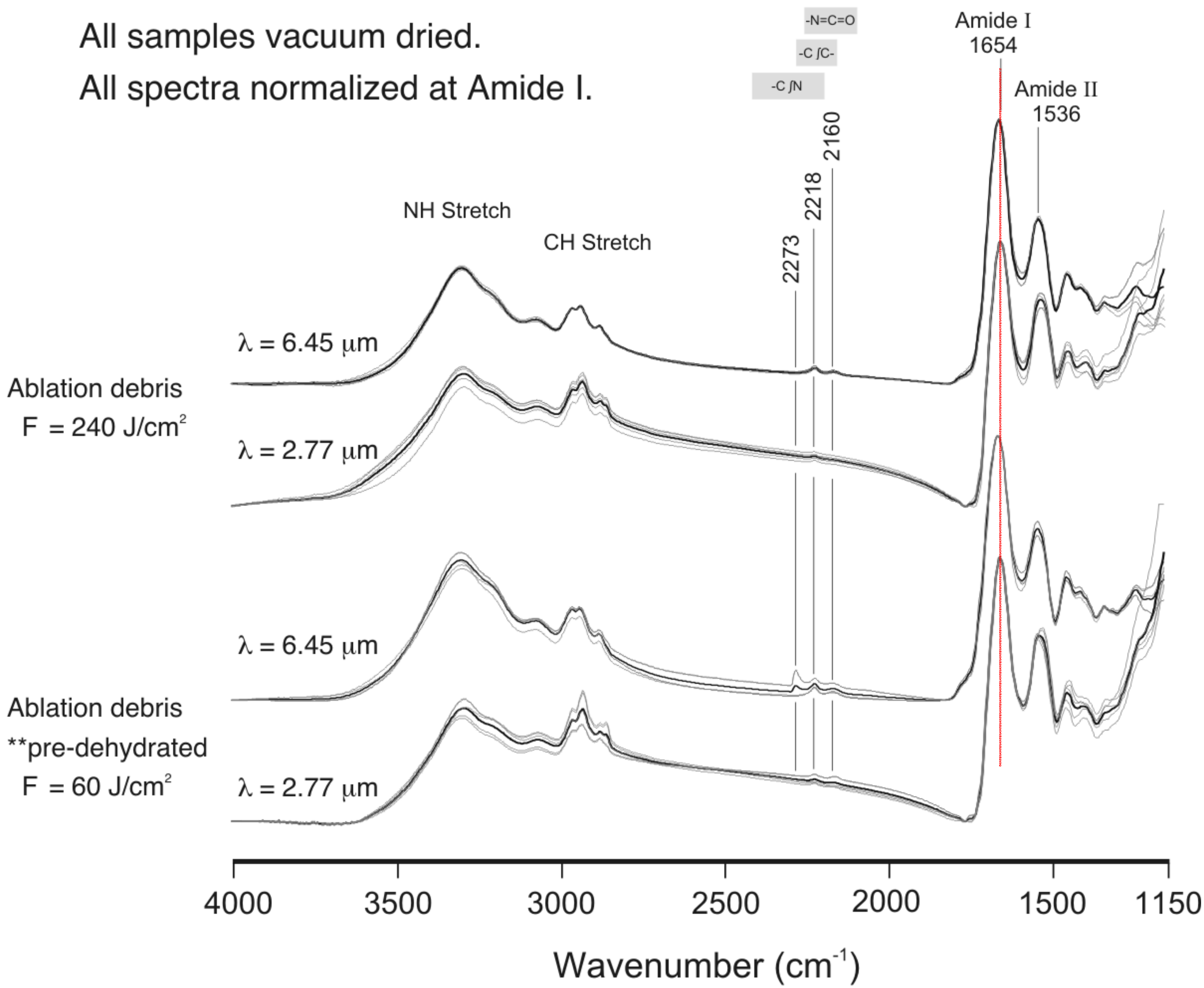
All samples vacuum dried.

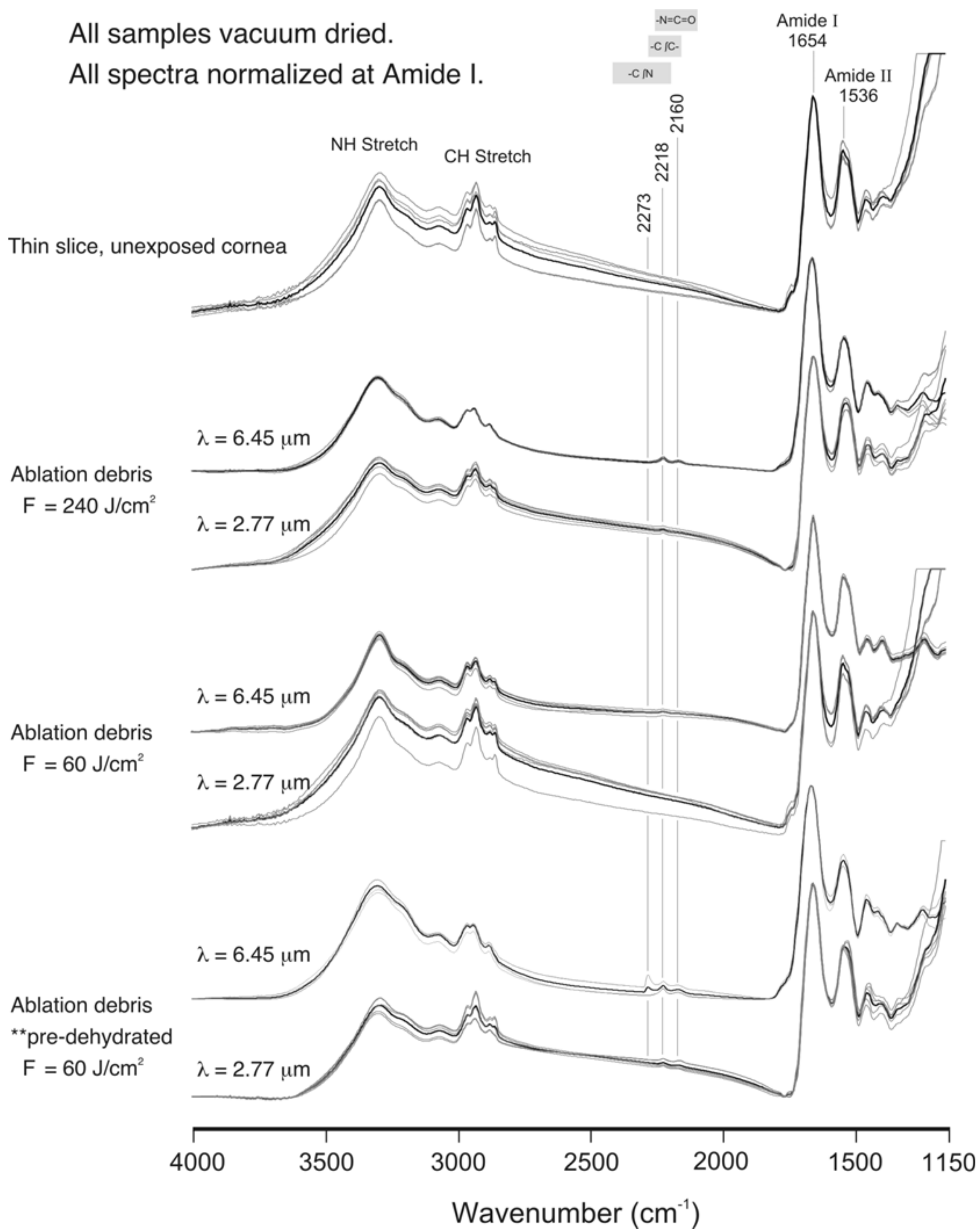
All spectra normalized at Amide I.



All samples vacuum dried.

All spectra normalized at Amide I.





Amide I frequency is NOT indicative of unfolded protein. For most samples it matches that of dry collagen triple helix. For others it is slightly upshifted.

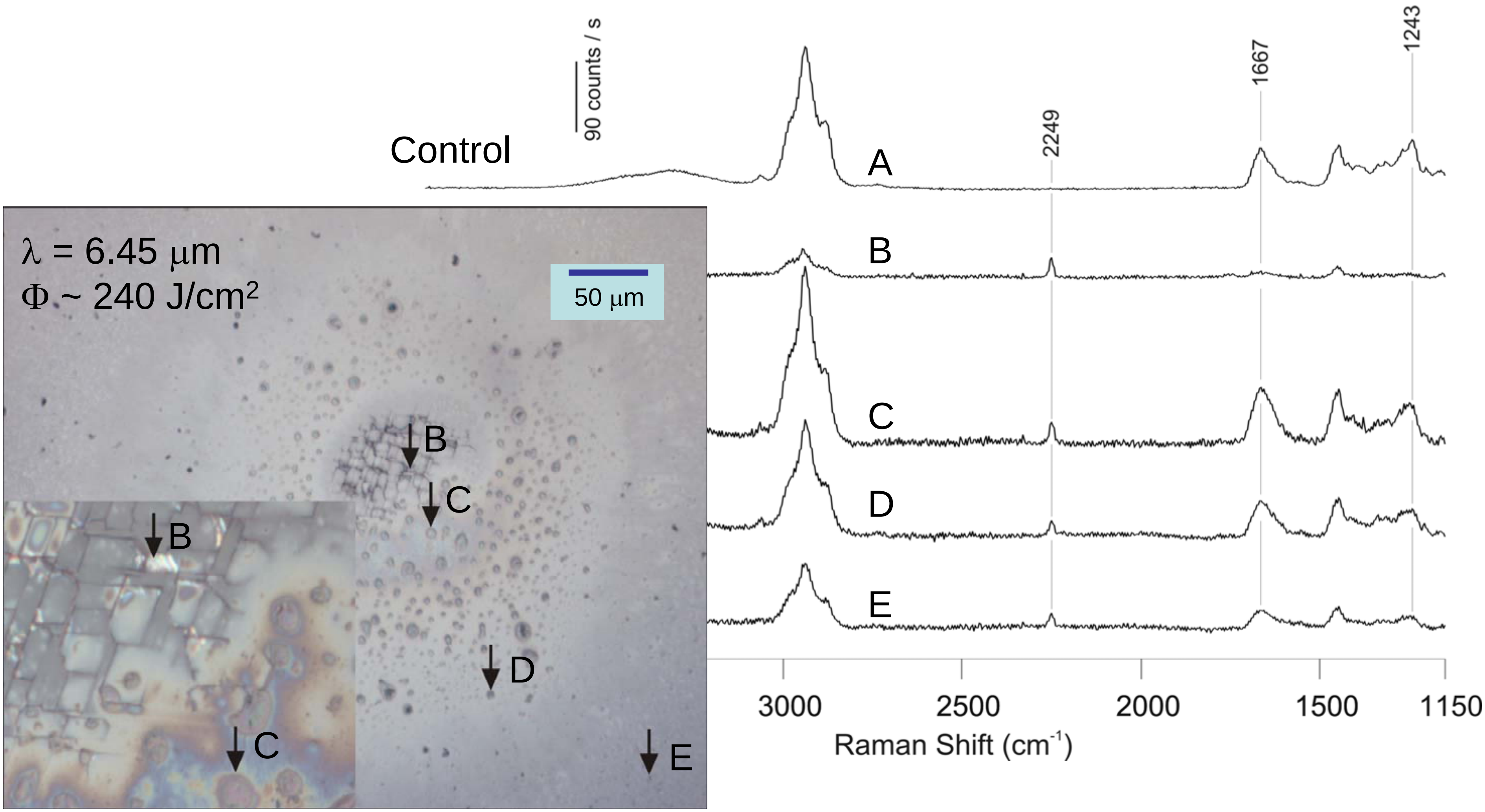
Similar ~2200 cm^{-1} bands observed on the surface of ablated bone, dentin and enamel. Previously assigned to cyanate ions.

- Dowker and Elliott, 1979, 1983; Spencer et al, 1996.

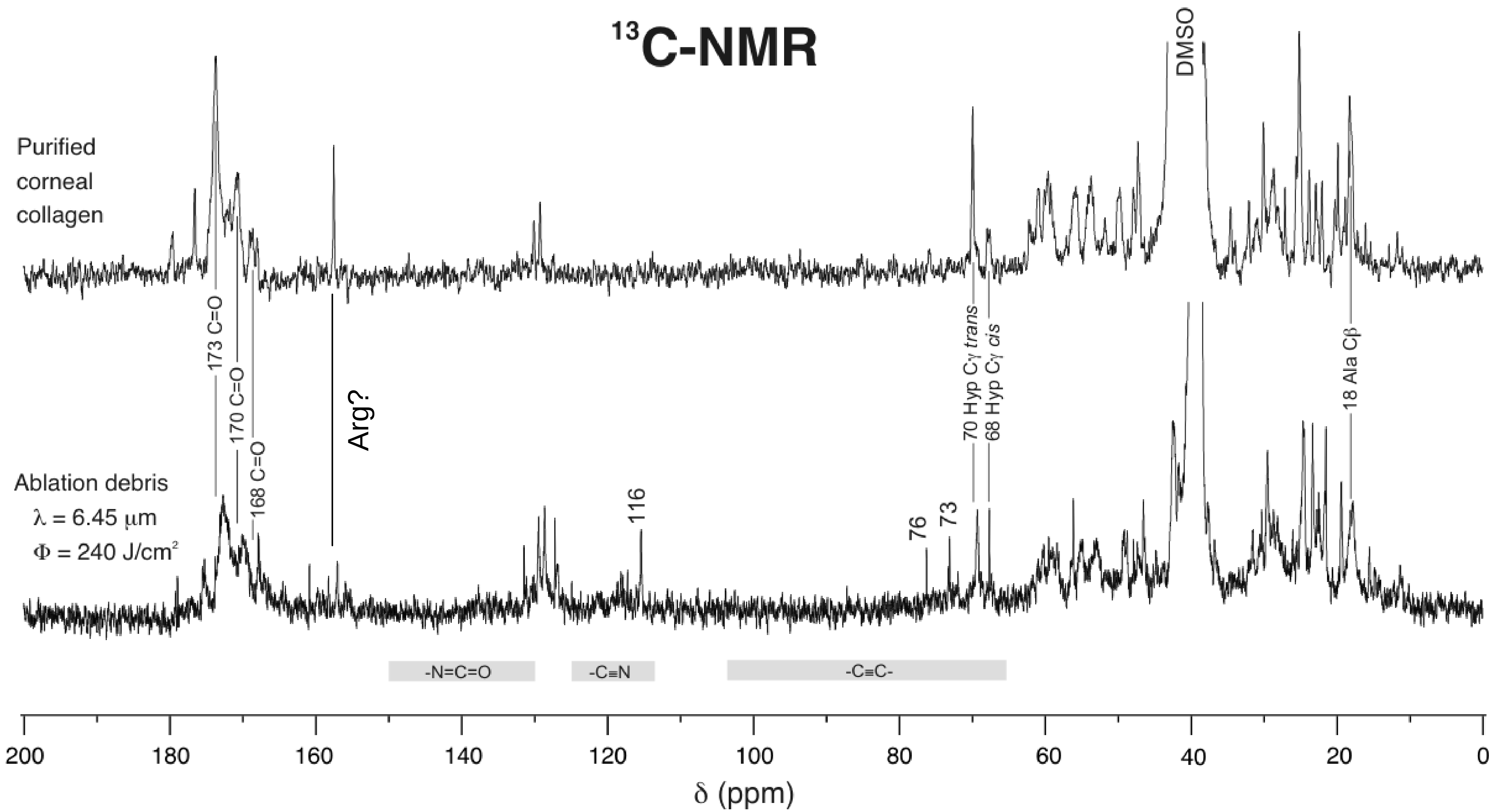
Similar bands arise in thermally treated dentin (>300 °C) and are accompanied by an ESR signal.

- Bachmann et al 2004.

Micro-Raman Spectra from SINGLE Macropulse Ablation



^{13}C-NMR

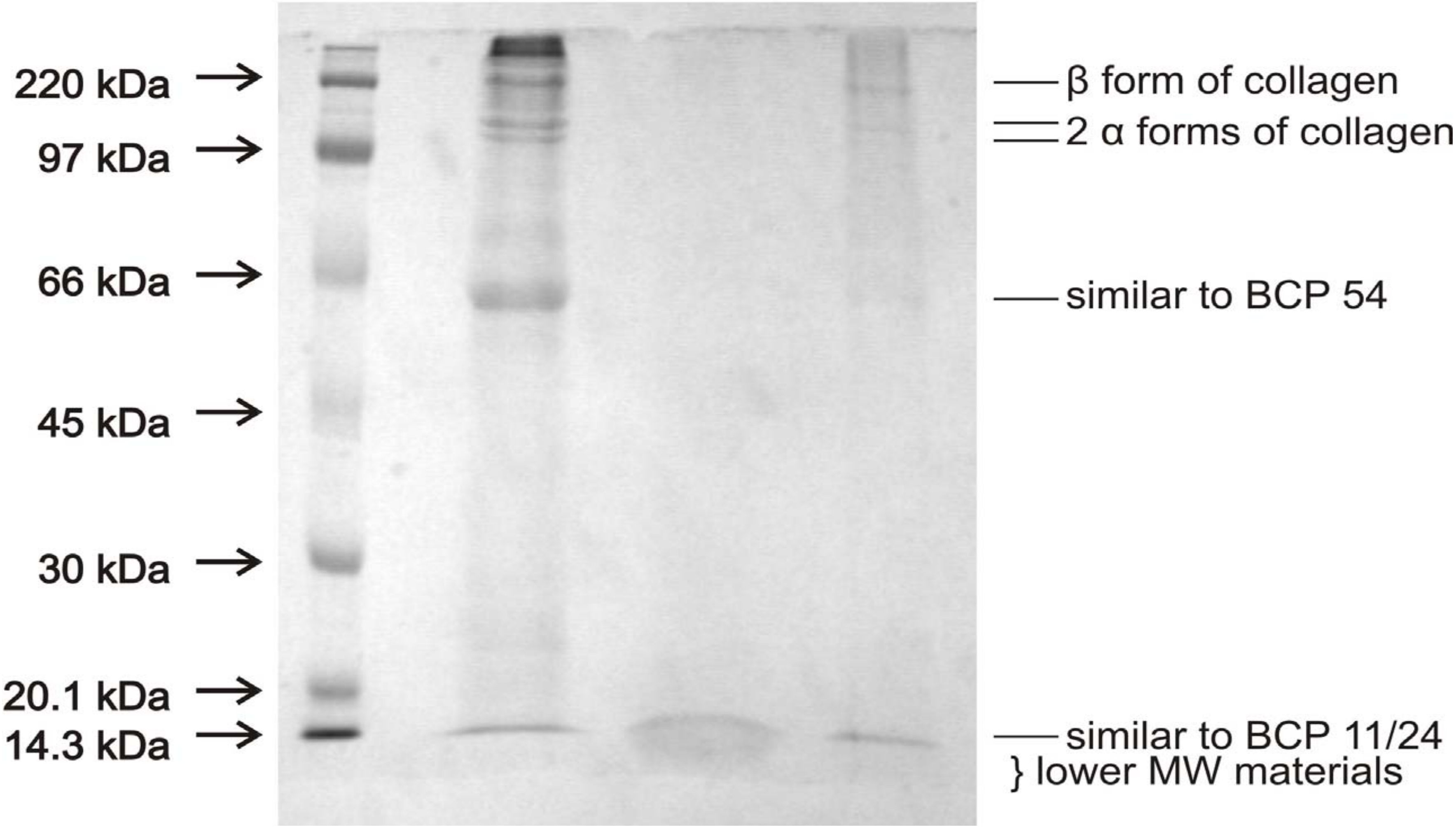


*Purified collagen was prepared using limited *pepsin A* digestion of porcine cornea.
**Ablation debris from ~200 CaF_2 windows was pooled.

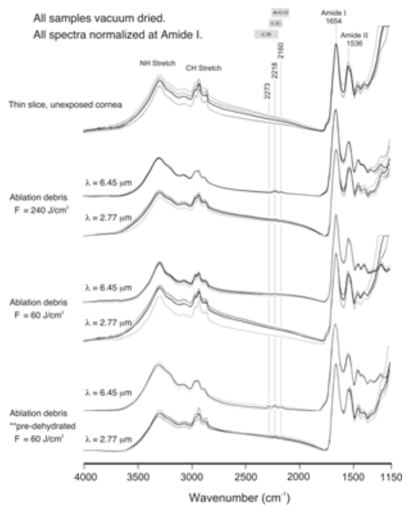
Band assignments from Saitô et al, 1984; Sarkar et al, 1984; Fujisawa et al, 1990; Saitô and Yokoi, 1992; Reichert et al, 2004

SDS (5%) extracts of:

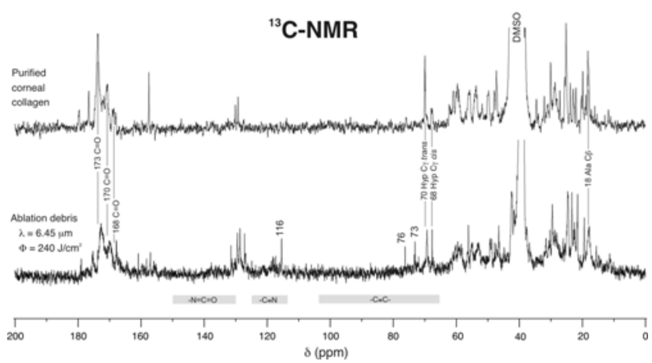
MW markers	Native cornea	Debris 6.45-µm 240 J/cm^2	Debris 2.77-µm 240 J/cm^2



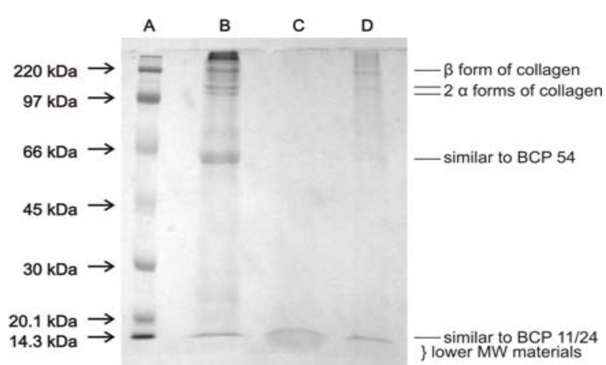
Band assignments from Cooper et al, 1990; Bakker et al 1992.



In the ablation debris, most of the primary and secondary structure of collagen remains intact.



But new chemical species (2-3) are also present. The combination of FTIR and ^{13}C-NMR definitively assigns one of the new species as a nitrile group (-C≡N) and suggests that the others could be alkynes (-C≡C-). The formation of these species is wavelength-, fluence- and hydration-dependent.



SDS-PAGE reveals that collagen is fragmented into smaller peptides in a λ-dependent manner. (~10 breaks per 1000 residue protein chain for λ = 6.45 μm)